J. Physiol. (1963), **165**, *pp.* 550–558
Printed in Great Britain

ENERGETICS OF SODIUM TRANSPORT IN *RANA PIPIENS*

BY ELISABETH DEE AND R. P. KERNAN
From the Biochemical Laboratory, University College, Dublin

(*Received* 7 *August* 1962)

The concept of a critical energy barrier to the excretion of Na by Na-rich muscle was first put forward by Conway (1960) to explain the fact that sartorii of *Rana temporaria*, made Na-rich by immersion during the night in cold K-free Ringer–Conway fluid (Boyle & Conway, 1941) containing 120 mM-Na, would not excrete Na in recovery fluid containing 10 mM-K unless the Na concentration of this fluid was reduced to 110 mM or less (Conway, Kernan & Zadunaisky, 1961).

An energy barrier as envisaged by Conway takes into account the osmotic and electrical gradients against which the Na ions must be moved during excretion from the muscle fibre, the total energy being represented by the equation,

$$dG/dn = RT.\ln([Na]_o/[Na]_i) + E_m F \quad (1)$$

where $[Na]_o$ and $[Na]_i$ represent the sodium concentrations in the recovery fluid and muscle fibre water respectively and E_m the membrane potential of the muscle fibres (outside minus inside) measured in the recovery fluid by the micro-electrode technique of Graham & Gerard (1946). A more comprehensive treatment of the energy equation above may be found elsewhere (Conway, 1960).

It was found (Conway *et al.* 1961) that if conditions of reimmersion of the Na-rich muscles was such as to produce an energy barrier greater than 2 cal/m-equiv Na, as happens when the recovery fluid contains 115 mM-Na or more with 10 mM-K, excretion of Na did not take place. On the other hand, if the energy requirements for excretion were reduced either by decreasing the Na concentration of the recovery fluid, thereby reducing the osmotic component of the energy barrier, or by increasing $[K]_o$ above 10 mM and thereby reducing membrane potential and associated electrical energy component of the barrier, Na excretion would take place up to a maximum of 22 m-equiv/kg wet wt. of muscle in a 2 hr recovery period. It was also shown that the lower the Na concentration of the recovery fluid the more Na would be excreted within certain limits. The critical energy barrier which is the maximum energy barrier against which Na excretion will take place can be measured either by increasing the Na concentration of the recovery fluid to the point where Na excretion

stops, and computing the energy barrier under these conditions, or by computing the energy barrier when Na excretion is complete at the end of the recovery period.

Recently Steinbach (1961) has reported that in the case of *Rana pipiens*, as distinct from *R. temporaria* used by us, the Na-rich sartorii will excrete Na without any necessity of reducing the Na concentration of the recovery fluid. In other words, he found that muscles immersed during the night in K-free Ringer's fluid with 125 or 115 mM-Na, reimmersed in the same fluid with addition of 10 mM-K (procedures referred to as 125, 0/125, 10 and 115, 0/115, 10) excreted Na in quantity. On the basis of these experiments he criticized the theory of the critical energy barrier. Because of this report and also to extend our study of the application of the theory to other tissues we have repeated with *R. pipiens* the type of experiments already reported by us for *R. temporaria* (Conway *et al.* 1961). In several important respects our experiments differed from those of Steinbach. First, the Ca concentration used by the latter in his Ringer's fluids was only 1×10^{-4} M compared with 9×10^{-4} M in our case. Our value is closer to that found in frog plasma (Boyle & Conway, 1941), but the lower Ca level facilitates enrichment of the muscles with Na, as will be shown later. The significance of the resulting higher Na concentrations in the muscles in Steinbach's experiments will be considered in the discussion. Secondly, in Steinbach's experiments membrane potential measurements were not made directly, but indirectly approximate values could be obtained through the Nernst equation. Here the membrane potentials were measured directly.

METHODS

The companion sartorius muscles of frogs of the *Rana pipiens* species imported from the U.S.A. were used in all experiments. These were first made Na-rich by immersion during the night in cold K-free Ringer–Conway fluid containing 120 mM-Na. Some muscles were analysed for Na and K immediately after this immersion, while their companions were reimmersed for 3 hr at 20° C in recovery fluid containing 5 or 10 mM-K. Here the Na excretion was estimated by a comparison of the mean Na concentration of the two sets of companion muscles. In other cases companion muscles were distributed between different reimmersion procedures. The recovery fluids used were based on the Ringer–Conway fluid and differed from the first immersion fluid only in the addition of K, and in changes in the Na and glucose concentrations. Where the NaCl concentration of the recovery fluid was reduced below the normal plasma level of 104 mM-Na, glucose was added to restore osmotic equilibrium. Where the external Na concentration was increased to 120 mM or more it was necessary to omit glucose from the medium. Where $[Na]_o$ was 125 or 130 mM, the Ringer's fluid was slightly hypertonic. For this reason a series of experiments was carried out to ensure that this hypertonicity did not interfere with Na excretion. This was done by adding glucose to recovery fluid in the 120, 0/120, 10 procedure, and comparing the Na content of muscles after 3 hr recovery in this fluid with that of companion muscles in the same procedure without the addition of glucose. Sufficient glucose was added to make the recovery fluid isotonic with recovery fluid containing 130 mM-Na.

The Na concentrations used in the recovery fluids were 130, 125, 120, 104 and 80 mM. The

composition of these fluids has already been reported (Conway *et al.* 1961). Some experiments were also carried out with low-Ca Ringer's fluids similar to those of Steinbach.

Chemical methods. The muscles for analysis were first blotted on moist filter paper to remove excess moisture. They were then carefully weighed and digested in hot concentrated pure HNO_3. About 1 ml. acid was required for each muscle. The average weight of the muscles used was 120 mg. When oxidation of the muscles was complete, after about 1 hr, the acid was evaporated and the residue taken up in 20 ml. of de-ionized water. This was then analysed by means of the Beckmann flame photometer. The mean water content of the muscles was estimated by drying some muscles to constant weight in an oven at 120° C before digesting, and by calculating the percentage weight change on drying.

The inter-fibre space of the muscles was measured for groups of three muscles, before and after recovery. An inulin dilution method was employed in which the muscles were immersed for from 2 to 4 hr in volumes of Ringer's fluid equal to the weight of the muscles, and containing a suitable concentration of inulin. After the required period of immersion the muscles were removed and the inulin concentration of the immersion fluid determined. Control solutions containing the same concentration of inulin, but to which muscles had not been added, and inulin standards were also set up. The inulin was estimated on the Beckmann spectrophotometer at 520 mμ following development of colour by heating the solutions at 78° C for 20 min with a HCl-resorcinol reagent (Higashi & Peters, 1950). The inter-fibre space of the muscles was calculated from the fall of inulin concentration in the solutions containing the muscles, compared with the control solutions.

Potential measurements. Membrane potentials of the muscles were measured by the microelectrode technique, the particular modification already described being used (Conway *et al.* 1961). About ten readings of potential were made on each muscle used. The readings were made about 5 min after reimmersion, when $[K]_o$ had distributed itself evenly through the inter-fibre space, and also at the end of the 3 hr recovery period. The muscles used for these measurements were later analysed for Na and K. Eight muscles were examined for each reimmersion procedure used. Over-all mean potentials, with standard errors were then calculated for all muscles in a particular set of conditions.

RESULTS

Inulin space. The inulin dilution was found to reach a plateau in about 1 hr, giving a mean inter-fibre space value of $16{\cdot}3 \pm 1{\cdot}4$ ml./100 g wet weight ± S.E. (16) for all muscles before and after reimmersion. There was no statistical difference in inter-fibre space after recovery of the muscles, so in computing the internal Na concentration of the muscle fibres the value of 16·3 ml./100 g was used. This is close to the value of $16{\cdot}7 \pm 0{\cdot}6$ found by Davenport & Alzamora (1962) for sartorii of *Rana pipiens* after 4 hr immersion with inulin. Conway, Harrington & Mullaney (1963) found an inter-fibre space of 12·7 ml./100 g in *R. temporaria* after soaking.

The total water content of the muscles averaged 80%. This gives us values of 16 and 64% for the extracellular and intracellular water, respectively. An allowance of 6 m-equiv Na/kg muscle was also made for sarcolemma Na (Conway & Carey, 1955), so in computing $[Na]_i$ the following equation was used,

$$[Na]_i = \frac{[Na] - [6 + [Na]_o \times 0{\cdot}16]}{0{\cdot}64}, \qquad (2)$$

where $[Na]_i$ and $[Na]_o$ are the concentrations in the muscle fibre water and recovery fluid respectively and [Na] is the concentration in the muscle expressed as m-equiv/kg wet wt. of tissue.

Effect of hypertonicity on Na excretion. On the basis of the assumption that 56 mM-NaCl is the approximate osmotic equivalent of 98 mM glucose (Hodgkin & Keynes, 1955), 17·5 mM glucose was added to the recovery fluid in the 120, 0/120, 10 procedure, making it isotonic with 130 mM-Na Ringer's fluid. One set of muscles was immersed in this fluid, while a set of companion muscles was immersed in 120 mM-Na Ringer's fluid without the addition of glucose. At the end of the 3 hr recovery period the former muscles had a mean Na content of $40·5 \pm 2·6$ (8) the latter $41·3 \pm 2·3$ (8), the difference being insignificant statistically ($P > 0·8$). It was concluded that the hypertonicity of the recovery fluid would not alter the Na excretion by the muscles.

TABLE 1. The effect of changes in the Na and K concentrations of the recovery fluid on the secretion of Na by Na-rich sartorii of *Rana pipiens*

Procedure	Mean Na content of muscles after reimmersion (m-equiv/kg ± S.E.)	Mean excretion of Na (m-equiv/kg)	Number of observations
120, 0/130, 10	$52·3 \pm 2·8$	$2·9 \pm 3·2$	12
120, 0/125, 10	$46·8 \pm 1·6$	$7·7 \pm 2·3$	18
120, 0/120, 10	$40·8 \pm 2·0$	$12·9 \pm 2·6$	16
120, 0/104, 10	$35·9 \pm 1·5$	$15·4 \pm 2·2$	21
120, 0/80, 10	$29·0 \pm 2·6$	$18·7 \pm 3·0$	18
120, 0/120, 5	$55·8 \pm 3·4$	None	12
	Mean Na content of muscles after first immersion		
	$53·7 \pm 1·6$		24

Allowance is made in Na excretion values above for changes of Na concentration in the inter-fibre spaces.

Effect of reduced Ca of the K-free and recovery fluids on the Na accumulation and excretion by the sartorii. In these experiments the 120, 0/120, 10 procedure was used. The Ringer–Conway fluid was used containing in one case the normal Ca concentration of 9×10^{4}M and in the other the concentration used by Steinbach, namely 10^{-4}M. In the first case the Na concentration of the muscles after the overnight immersion in K-free Ringer's fluid was $53·7 \pm 1·6$ (24) m-equiv/kg and in the lower-Ca fluid the muscles contained $63·4 \pm 2·4$ (7) m-equiv Na/kg after this immersion. The Na concentration in the muscles after recovery were $40·8 \pm 2·0$ (16) and $52·0 \pm 3·3$ (7) m-equiv/kg respectively.

Effect of external Na concentration on the Na excretion from Na-rich sartorii after 3 hr reimmersion in recovery fluid containing either 10 *or* 5 mM-*K*. The results for this series are shown in Table 1. It is evident from

the table that the amount of Na excreted depends on the Na concentration of the recovery fluid. As $[Na]_o$ is increased to 130 mM the excretion of Na is reduced to an insignificant level. The results in Table 1 also show that reduction of $[K]_o$ from 10 to 5 mM also eliminated excretion. The Na concentration in the sartorii after the first immersion was $53{\cdot}7 \pm 1{\cdot}6$ m-equiv/kg for *R. pipiens*, compared with $59{\cdot}0 \pm 0{\cdot}7$ for *R. temporaria* (Carey, Conway & Kernan, 1959). These values are low compared with those reported by Steinbach (1961), using low-Ca Ringer's fluid, namely $70{\cdot}0 \pm 1{\cdot}4$ in 115 mM-Na and $75{\cdot}0 \pm 2{\cdot}3$ m-equiv/kg in 125 mM-Na Ringer's fluid. The maximum amount of Na excreted in our experiments (Table 1) was 18·7 m-equiv/kg muscle, which was low compared with Steinbach's results, but the lowest Na level reached in the muscles after recovery was only 29 m-equiv/kg in our case compared with 44 m-equiv/kg with the latter.

TABLE 2. Energy barriers to Na excretion from Na-rich sartorii measured soon after reimmersion

Procedure	Membrane potential (mV ± S.E.)	Energy barrier (cal/m-equiv Na) $E_m F$	$RT.\ln([Na]_o/[Na]_i)$	Total	Mean Na excretion (m-equiv/kg)
120, 0/130, 10	$71{\cdot}5 \pm 0{\cdot}4$ (85)	1·65	0·62	2·27	$2{\cdot}9 \pm 3{\cdot}2$
120, 0/125, 10	$72{\cdot}0 \pm 0{\cdot}4$ (61)	1·66	0·59	2·25	$7{\cdot}7 \pm 2{\cdot}3$
120, 0/120, 10	$67{\cdot}4 \pm 0{\cdot}4$ (81)	1·55	0·57	2·12	$12{\cdot}9 \pm 2{\cdot}6$
120, 0/104, 10	$63{\cdot}8 \pm 0{\cdot}5$ (64)	1·47	0·49	1·96	$15{\cdot}4 \pm 2{\cdot}2$
120, 0/80, 10	$62{\cdot}6 \pm 0{\cdot}4$ (67)	1·44	0·34	1·78	$18{\cdot}7 \pm 3{\cdot}0$
120, 0/120, 5	$83{\cdot}6 \pm 0{\cdot}4$ (84)	1·92	0·57	2·49	None

Energy barrier to Na excretion at the beginning of reimmersion compared with the amount of Na excreted. In Table 2 are shown the mean membrane potentials of the muscles measured about 5 min after reimmersion of the Na-rich muscles in recovery fluid. The electrical energy barriers calculated from the potentials are also shown. The fall of potential observed on reduction of $[Na]_o$ is possibly associated with reduction of external Cl^-.

In column 5 of Table 2 are shown the total energy barriers, that is the sum of the electrical and osmotic energy gradients against which the Na must be moved during excretion. If one compares the total energy barriers shown with the amount of Na excreted by the muscles, it seems that the critical energy barrier above which no Na excretion takes place lies between 2·27 and 2·49 cal/m-equiv Na. It also appears from this Table that the lower the energy barrier in column 5 the more Na is excreted by the muscles.

Energy barriers at the conclusion of Na excretion. A more precise evaluation of the critical energy barrier may be obtained if one examines conditions at the conclusion of Na excretion by the muscles. In Table 3 are shown the electrical, osmotic and total energy barriers at the end of the

3 hr reimmersion, when the internal Na of the muscles has fallen to steady value. By this method it seems that the total energy barrier at the conclusion of Na excretion, which could be regarded as the critical energy barrier to Na excretion, lies somewhere in the range from 2·39 to 2·50 cal/m-equiv Na for the various reimmersion procedures, giving a mean value of 2·44 cal/m-equiv Na. This value lies within the range of 2·27–2·49 cal/m-equiv Na indicated by Table 2. The value of 2·4 cal/m-equiv Na found here with *R. pipiens* is higher than the value already found for the critical energy barrier in *R. temporaria*, namely 2·1 cal/m-equiv Na (Conway *et al.* 1961), indicating a greater ability to excrete Na in the former species.

TABLE 3. Energy barriers at the conclusion of Na excretion by the Na-rich sartorii of *Rana pipiens*

Procedure	Na in muscle fibre water (m-equiv/l.)	Membrane potential (mV ± S.E.)	Energy barrier (cal/m-equiv Na) $RT.\ln([Na]_o/[Na]_i)$	$E_m F$	Total
120, 0/130, 10	40·0	73·8 ± 0·6 (84)	0·69	1·71	2·40
120, 0/125, 10	32·6	69·6 ± 0·7 (80)	0·78	1·61	2·39
120, 0/120, 10	24·4	68·7 ± 0·7 (90)	0·92	1·58	2·50
120, 0/104, 10	20·8	66·2 ± 0·5 (65)	0·93	1·53	2·46
120, 0/80, 10	15·9	65·0 ± 0·3 (64)	0·94	1·50	2·44

Mean critical energy barrier 2·44 cal/m-equiv Na.

DISCUSSION

The concept of the critical energy barrier to Na excretion in Na-rich frog muscle requires that the following criteria shall be obeyed:

(*a*) At the critical energy barrier no excretion should take place, but *decrease* of $[Na]_o$ with $[K]_o$ maintained constant should give rise to a marked excretion of Na.

(*b*) At the critical barrier, *increasing* $[K]_o$ with $[Na]_o$ constant should also bring about Na excretion.

(*c*) Where Na excretion is already taking place in the presence of a relatively low energy barrier, raising $[Na]_o$ or lowering $[K]_o$ should reduce and eventually abolish the excretion.

(*d*) The increased excretion caused by lowering $[Na]_o$ or raising $[K]_o$ should come to an end when the original critical energy barrier is reached.

When these criteria are fulfilled it may be concluded that a critical energy barrier exists in a particular tissue and that this barrier is the main controlling factor in Na excretion.

That these criteria are satisfied for the sartorii of *Rana pipiens* is shown here. In Table 1, decrease of $[Na]_o$ below 130 mM, with constant $[K]_o$, initiates Na excretion. Decreasing $[K]_o$ from 10 to 5 mM with $[Na]_o$

constant abolishes Na excretion (Table 1). When secretion is already present, increasing $[Na]_o$ in stages (Table 1) reduces and finally abolishes the exit of Na from the muscles. Finally, when secretion is taking place as in the 120, 0/104, 10 and 120, 0/80, 10 procedures, and is allowed to go to completion, the energy barriers, originally 1·96 and 1·78, increase to 2·46 and 2·44 cal/m-equiv Na, respectively. The mean critical energy barrier measured at the conclusion of excretion for all procedures was 2·44 cal/m-equiv Na, which is within the range indicated in Table 2, namely 2·3–2·5 cal/m-equiv Na, the *original* critical energy barrier referred to (*d*) in the criteria above.

The results in Table 1 indicate that while there is indeed some Na excretion in the 120, 0/120, 10 procedure, as suggested by Steinbach's experiments, a further loss of 8·5 m-equiv Na/l. fibre water takes place when $[Na]_o$ is reduced to 80 mM, the intrafibre Na reaching a concentration of 15·9 m-equiv/l. So, contrary to the opinion expressed by Steinbach (1961), there is a relationship between $[Na]_o$ and the amount of Na excreted.

An explanation of the large amounts of Na accumulated in Steinbach's experiments would seem to be his use of low Ca concentrations in his Ringer's fluids. We have shown here that lowering of Ca concentration in our Ringer's fluid leads to a greater enrichment of the muscles with Na during the first immersion, but also leads to less complete recovery of the muscles on reimmersion, with resulting fall of the critical energy barrier. It has already been shown (Kernan, 1962) that addition to the recovery fluid of insulin and lactate while duplicating more closely the composition of normal frog plasma can also have the effect of increasing the critical energy barrier. On the other hand, reduction of Ca below its normal plasma concentration (Steinbach, 1961) might be expected to reduce the critical energy barrier somewhat, resulting in less complete recovery of the Na-rich sartorii on reimmersion. For example, after the 115, 0/115, 10 and 125, 0/125, 10 experiments of Steinbach the Na concentration in the muscles were 47 ± 2 and 51 ± 4 m-equiv/kg, respectively, compared with $40{\cdot}8 \pm 2{\cdot}0$ m-equiv/kg in our 120, 0/120, 10 procedure (Table 1).

While we would agree with Steinbach that it is possible with *Rana pipiens* to demonstrate Na excretion without the necessity of reducing the Na concentration of the recovery fluid below that used in the first immersion, this in no way invalidates the concept of the critical energy barrier. A consideration of equation 1, for example, will show that one may reduce the energy barrier to Na excretion either by reducing $[Na]_o$ or by increasing $[Na]_i$ with $[K]_o$ kept constant. In our earlier work (Conway *et al.* 1961) we varied $[Na]_o$ keeping $[Na]_i$ constant after the first immersion.

Steinbach, by very high loading of the muscles with Na, and by the use

of a wide range of $[Na]_i$ values, adopted the latter method of reducing the energy barrier, keeping $[Na]_o$ unchanged on reimmersion. So even if the critical energy barrier was not higher in *R. pipiens* than in *R. temporaria* he would still have no difficulty in demonstrating Na excretion without reducing $[Na]_o$. In one of Steinbach's experiments he soaks muscles for 2 hr at room temperature in 130 mM-Na, K-free Ringer's fluid, resulting in one case in a final concentration of 130 m-equiv Na/kg wet wt. muscle. In this case there must be no osmotic energy barrier to Na excretion on reimmersion in Ringer's fluid containing 130 mM-Na and 10 mM-K, so it is not surprising that there is considerable Na excretion here without reduction of $[Na]_o$.

On the basis of the experiments reported here it is concluded that there exists for *R. pipiens*, as already found for *R. temporaria*, a critical energy barrier to Na excretion, and that its value is 2·4 cal/m-equiv Na compared with 2·1 in the latter species (Conway *et al.* 1961).

SUMMARY

1. Na excretion from sartorii of *Rana pipiens* was measured after enrichment of the muscles by immersion during the night in cold K-free Ringer–Conway fluid with 120 mM-Na, and reimmersion for 3 hr at 20° C in the same fluid containing 10 or 5 mM-K, and different concentrations of Na ranging from 80 to 130 mM. Membrane potential measurements were made at the beginning and end of reimmersion.

2. At the end of the first immersion the Na of the muscles rose to $53{\cdot}7 \pm 1{\cdot}6$ m-equiv/kg wet wt. of muscle, compared with a value of $70{\cdot}0 \pm 1{\cdot}4$ m-equiv/kg reported by Steinbach (1961). The difference is interpreted as due to his use of a much lower Ca concentration in the Ringer's fluid.

3. No significant Na excretion took place in recovery fluid containing 130 mM-Na and 10 mM-K, but as the $[Na]_o$ was lowered to 125, 120, 104 and 80 mM the amount of Na excreted increased progressively. No excretion took place in the 120, 0/120, 5 procedure. The lowest intracellular Na concentration reached was 15·9 m-equiv/l. fibre water in 80 mM-Na recovery fluid, a loss of 28·6 m-equiv Na/l.

4. The energy barrier to Na excretion was calculated for the various reimmersion fluids, and the critical energy barrier above which no excretion of Na would take place was found to be 2·4 cal/m-equiv Na, compared with 2·1 already reported for *R. temporaria* (Conway *et al.* 1961).

5. The results obtained here are discussed in the light of Steinbach's (1961) attack on the concept of a critical energy barrier to Na excretion in frog skeletal muscle (Conway, 1960; Conway *et al.* 1961).

We are grateful to Professor E. J. Conway, F.R.S. for helpful discussion, and to the Medical Research Council of Ireland for grants-in-aid. The work was supported in part by a research grant (H–4860) from Physiology Division Public Health Service, N.I.H.

REFERENCES

BOYLE, P. J. & CONWAY, E. J. (1941). Potassium accumulation in muscle and associated changes. *J. Physiol.* **100**, 1–63.

CAREY, M. J., CONWAY, E. J. & KERNAN, R. P. (1959). Secretion of sodium ions by the frog's sartorius. *J. Physiol.* **148**, 51–82.

CONWAY, E. J. (1960). Critical energy barriers in the excretion of sodium. *Nature, Lond.*, **187**, 394–396.

CONWAY, E. J. & CAREY, M. J. (1955). Muscle sodium. *Nature, Lond.*, **175**, 773.

CONWAY, D. M., HARRINGTON, M. G. & MULLANEY, M. (1963). The nature of sodium exchanges in isolated frog sartorii. *J. Physiol.* **165**, 246–265.

CONWAY, E. J., KERNAN, R. P. & ZADUNAISKY, J. A. (1961). The sodium pump in skeletal muscle in relation to energy barriers. *J. Physiol.* **155**, 263–279.

DAVENPORT, H. W. & ALZAMORA, F. (1962). Sodium, potassium and water in frog gastric mucosa. *Amer. J. Physiol.* **202**, 711–715.

GRAHAM, J. & GERARD, R. W. (1946). Membrane potentials and excitation of impaled single muscle fibres. *J. cell. comp. Physiol.* **28**, 99–117.

HIGASHI, A. & PETERS, L. (1950). A rapid colorimetric method for the determination of inulin in plasma and urine. *J. Lab. clin. Med.* **35**, 475–482.

HODGKIN, A. L. & KEYNES, R. D. (1955). Active transport of cations in giant axons of *Sepia* and *Loligo*. *J. Physiol.* **128**, 28–60.

KERNAN, R. P. (1962). The role of lactate in the active excretion of sodium by frog muscle. *J. Physiol.* **162**, 129–137.

STEINBACH, H. B. (1961). Na excretion by the sartorius of *Rana pipiens*. *J. gen. Physiol.* **44**, 1131–1142.